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REVIEW

Nanotechnology for COVID-19: Therapeutics and Vaccine Research

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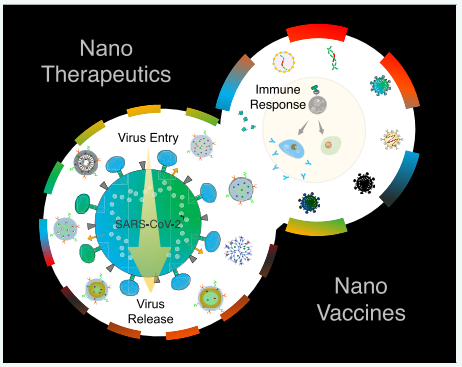
ABSTRACT: **The current global health threat by the novel coronavirus disease 2019 (COVID-19) requires an urgent deployment of advanced therapeutic options available. The role of nanotechnology is highly relevant to counter this "virus" nano enemy. Nano intervention is discussed in terms of designing effective nanocarriers to counter the conventional limitations of antiviral and biological therapeutics. This strategy directs the safe and effective delivery of available therapeutic options using engineered nanocarriers, blocking the initial interactions of viral spike glycoprotein with host cell surface receptors, and disruption of virion construction. Controlling and eliminating the spread and reoccurrence of this pandemic demands a safe and effective vaccine strategy. Nanocarriers have potential to design risk-free and effective immunization strategies for severe acute respiratory syndrome coronavirus 2 vaccine candidates such as protein constructs and nucleic acids. We discuss recent as well as ongoing nanotechnology-based therapeutic and prophylactic strategies to fight against this pandemic, outlining the key areas for nanoscientists to step in.**



KEYWORDS: *coronavirus disease 2019, severe acute respiratory syndrome coronavirus 2 structure, immunopathology, nanomedicine, targeted therapeutics, combination drug delivery, vaccine nanocarriers, vaccine adjuvant nanoparticles, repurposed nanotechnology*

The novel coronavirus now officially termed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the COVID-19 disease outbreaks.[1,2] Wuhan (China) was the first epicenter of this pandemic, and now with more than 8.3 million reported infections and over 449,099 deaths as of June 17, 2020, it is considered as the worst crisis since World War II.[3−5] The worldwide impact of this pandemic is frightening, and it might not have reached the pinnacle yet. The human race is also facing a crisis situation due to mandatory quarantines and lockdowns.[6,7] The world economy is already facing a long-lasting dent, and the situation will surely worsen if the viral spread is not controlled.[8,9]

Alpha, beta, gamma and delta are the four classes of the coronavirus (CoV) family, all featuring a single-stranded positive-sense RNA genome. The membrane envelops encapsulating the viral genome are decorated with glycoprotein spike transmembrane proteins. The word "coronavirus" is named for the club-shaped protein spikes on their surface when viewed under a transmission electron microscope (TEM). The causative agent behind the COVID-19 pandemic belongs to the beta class.[10] The same coronavirus class was responsible for the severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS).[11] Fever, dry cough, fatigue, and difficulty in breathing are among the initial symptoms of a SARS-CoV-2 infected patient.[12] This is the more contagious virus in its class and majorly affects the lower respiratory system initiating viral pneumonia. Vital organs including cardiac, liver, kidneys, gastrointestinal tract (GIT), and the central nervous system (CNS) may also be affected, causing multiple organ complicacies.[13−20]

LIFE CYCLE, PATHOPHYSIOLOGY, AND STRUCTURE

SARS-CoV-2 has a single-stranded RNA genome of approximately 34 kilobases and a nucleocapsid of helical symmetry. The SARS-CoV-2 genome is 80% identical to the SARS-CoV and 96% to the BatCoV RaTG13.[10] The integrity of the SARS-CoV particle is maintained by four proteins: (i) The S protein (Spike glycoprotein) that enables the attachment of the virus to host cells followed by membrane fusion, hence, promoting

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the entry of SARS-CoV into the host cells; (ii) the abundant M protein (membrane) that maintains the membrane integrity of the viral particle; (iii) the E protein (envelope) is the smallest protein and plays a structural role and helps in assembly and budding; and (iv) the N protein (nucleocapsid) predominantly binds to the SARS-CoV RNA and supports nucleocapsid formation.[21−27] The angiotensin-converting enzyme 2 (ACE2) is the key receptor for entry of SARS-CoV-2 in the cells of the host. Cellular proteases [human airway trypsin-like protease and cathepsins and transmembrane protease serine 2 (TMPRSS2)] control the viral entry mechanism by splitting the spike protein and initiating further penetration mechanisms.[28] At least six open reading frames are present in a typical CoV genome that encode for the production of subgenomic RNAs, 16 nonstructural proteins (nsps), and structural proteins (spike, membrane, envelope, and nucleocapsid protein).[29−31] The life cycle of SARS-CoV-2 explaining the entire pathophysiology mechanism is detailed in (Figure 1, I).

Coronavirus S proteins promote the entry of the virus into host cells and are the area of focus for various antibodies. The surface S protein (spike glycoprotein) of virions is the site for recognition and membrane fusion.[32−34] The S protein (a trimer) gets cleaved into S1 and S2 subunits. The S1 subunits contain the receptor binding domain (RBD) and are released in post-transfusion conformation.[34−37] S1 directly binds to the peptidase domain (PD) of the ACE2, while S2 subunits help in the membrane fusion that is critical for viral infection.[38,39] S2 contains cleavage sites and is sliced by host proteases.[35,40,41]

ACE2 is a dimer of the two units and accommodates the RBD in its peptidase domain. The contact between the ACE2 and SARS-CoV-2 is facilitated by polar interactions.[37,38,42] An arch-shaped helix of the peptidase domain of ACE2 interacts with the loop region of the RBD of the S protein (Figure 1, II). The other helix and loops connect the antiparallel strands and coordinate the peptidase domain to the RBD. The amino acid interactions that are observed in RBD of SARS-CoV-2 and the peptidase domain of ACE2 are considered important aspects for the inhibitor design.[43] It was observed that the amino acid GLN498 of SARS-CoV-2 interacts with ACE2 at the ASP38, TYR41, GLN42, LEU45, and LYS353 amino acids, while LEU455 of the virus has interaction with ASP30, LYS31, and HIS34. More interactions include the SARS-CoV-2, PHE486 with GLN24, LEU79, MET82, TYR83, and LEU472. GLN493 showed interaction with ACE2 LYS31 and HIS34 and forms an H-bond with GLU35. The amino acid ASN501 has a similar type of interaction with ACE2 LYS353, GLY354, and ASP355, while H-bond interaction is observed with TYR41.[44] The binding affinity of the RBD domain of SARS-CoV-2 and PD of ACE2 is higher when compared to SARS-CoV.[43] It was reported that in SARS-CoV-2 the amino acid LYS417 showed a salt bridge interaction with ASP30 of ACE2. The positive charged patch contributed toward the electrostatic potential on the surface of RBD that is added by LYS417 in SARS-CoV-2 and absent in SARS-CoV.[43,45,46]

Examination of the SARS-CoV-2 virion architecture using TEM reveals a roughly spherical or moderately pleiomorphic morphology. The virion diameter is observed to have a broad distribution of 80−160 nm and a condensed mass of nucleic acid and nucleocapsid protein underneath a well-defined lipid bilayer envelop.[47] TEM also reveals the nail-like shape of the SARS-CoV-2 spikes with a 7 nm wide head and a 23 nm long body. After the dissociation of the S1 subunit from the S

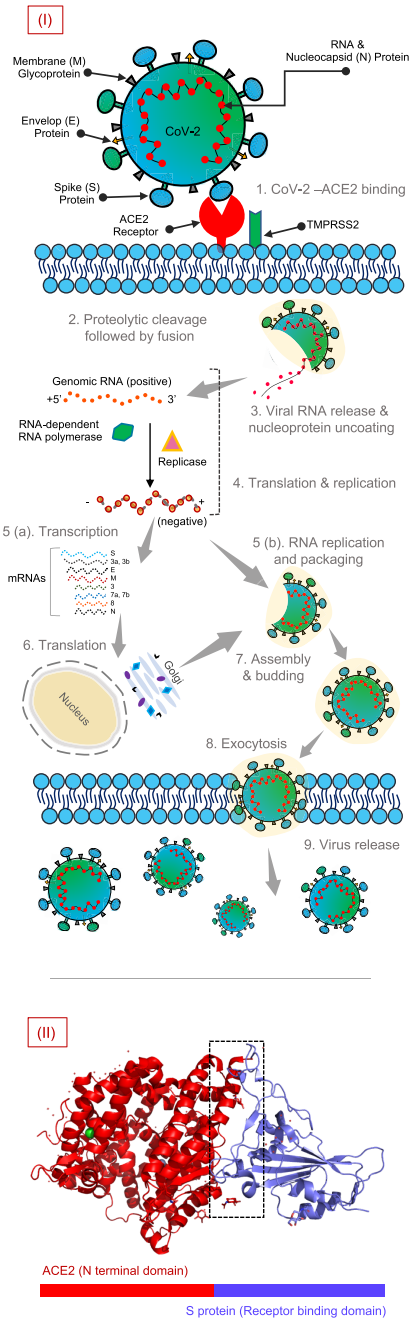

Figure 1. SARS-CoV-2 structure and pathophysiology. (I) SARS-CoV-2 life cycle: The viral S protein binds to the ACE2 receptor of the host. Following the entry, there is the proteolytic cleavage of the virus envelope ensuing in the release of genomic RNA in the cytoplasm, and smaller RNAs ("subgenomic mRNAs") are made. These mRNAs are translated to several proteins (S, M, N, *etc.*) essential for the construction of viral assembly. S, E, and M proteins enter the endoplasmic reticulum (ER), and nucleoprotein complex formation occurs from the combination of nucleocapsid (N) protein and genomic RNA (positive strand). Formation of the complete virus particle (proteins and genome RNA assembly) occurs in ER-Golgi apparatus compartment. Virus particles are then transported and released *via* vesicles formation and exocytosis. (II) ACE2-RBD (S protein): A single unit of peptidase domain of human ACE2 (red) interacting with the RBD of the S protein (blue), (boxed region represents the amino acid interactions sites).

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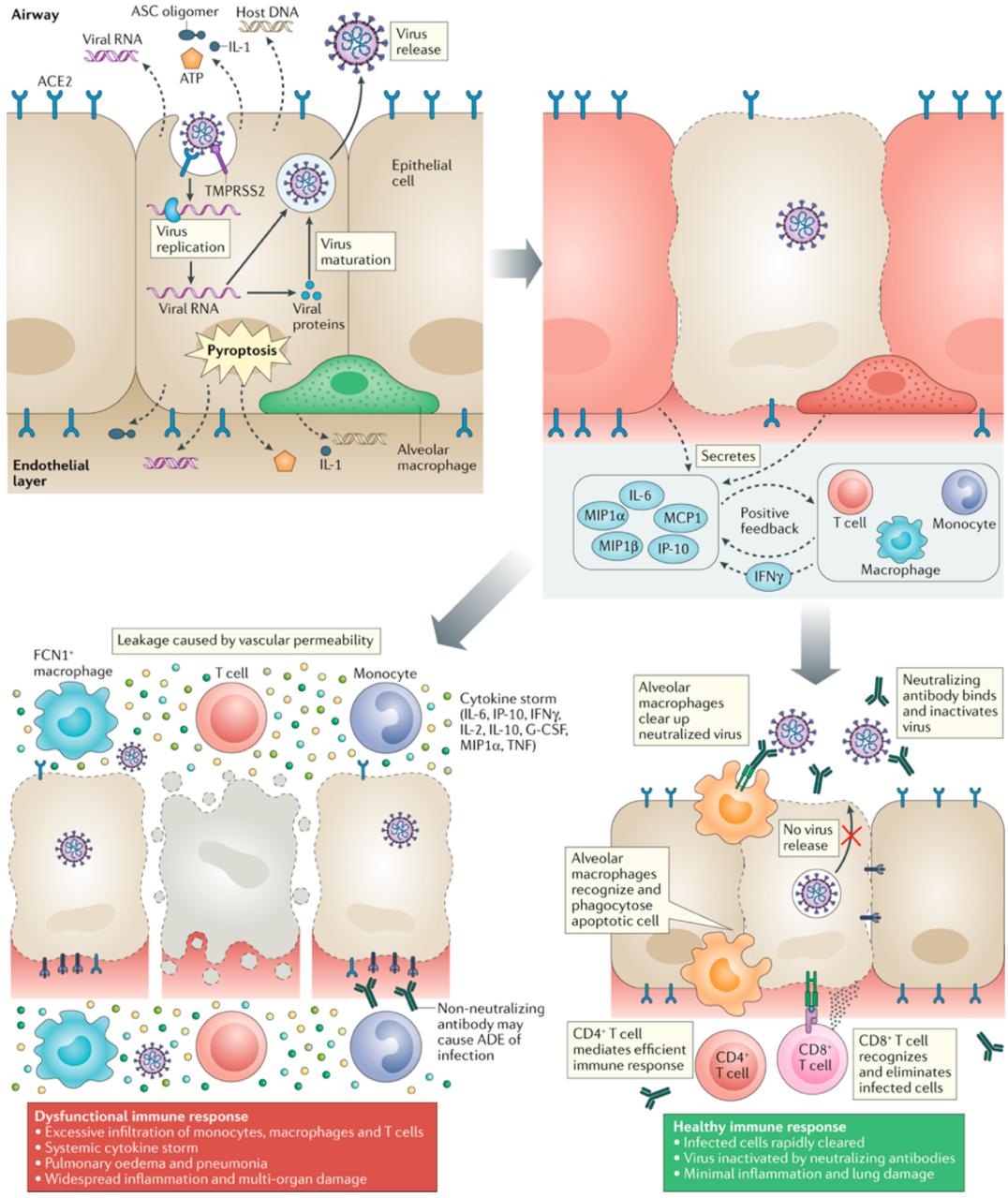

Figure 2. Healthy and dysfunctional immune response during SARS-CoV-2 infection. A virus-infected cell undergoes pyroptosis and generates molecules (including damage-associated molecular patterns, nucleic acids, ASC oligomers, and ATP) to trigger neighboring epithelial and endothelial cells and macrophages. Pro-inflammatory proteins (cytokines and chemokines) released there migrate the T cells, monocytes, and macrophages to the infection site. A loop of pro-inflammatory feedback is started by IFNγ (released by T cells). The healthy immune response following this initial inflammation is comprised of T cell-mediated elimination of the infected cells, neutralizing antibody-mediated (produced by B cells) viral inactivation, macrophage-dependent recognition, and clearance of apoptotic cells by phagocytosis. However, excessive infiltration of immune cells and the resulting cytokine storm leads to a dysfunctional immune response (*i.e.*, multiorgan damage). Antibody-dependent enhancement (ADE) of the viral infection may occur as a result of non-neutralizing antibody production by B cells.[61] Adapted with permission from ref 61. Copyright 2020 Springer Nature.

protein, a conformational change was observed in the S2 subunit. This change from a compressed form to a nail-like shape was confirmed by different researchers and is called a postfusion state. A three-dimensional (3D) map and two-dimensional projection images of S2 protein at the postfusion state were provided by Song *et al.* with negative staining EM.[37] It was also confirmed from biophysical assays and Cryo-EM structure analysis that SARS-CoV-2 S protein binds at least 10 times more tightly to ACE2 host cell receptors when compared to the spike protein of SARS-CoV.[39,43,48]

IMMUNOPATHOLOGY (INNATE AND ADAPTIVE IMMUNE RESPONSE)

Both innate and adaptive immune responses are initiated by the SARS-CoV-2 infection. Humoral and cell-mediated immune responses are further reported to play a protective role against the infection. Whereas, inflammation and dysfunctional immune responses result in both local and systemic tissue damage.[49−52] Pathogen-associated molecular patterns (PAMPs) recognition triggers the innate immune response,

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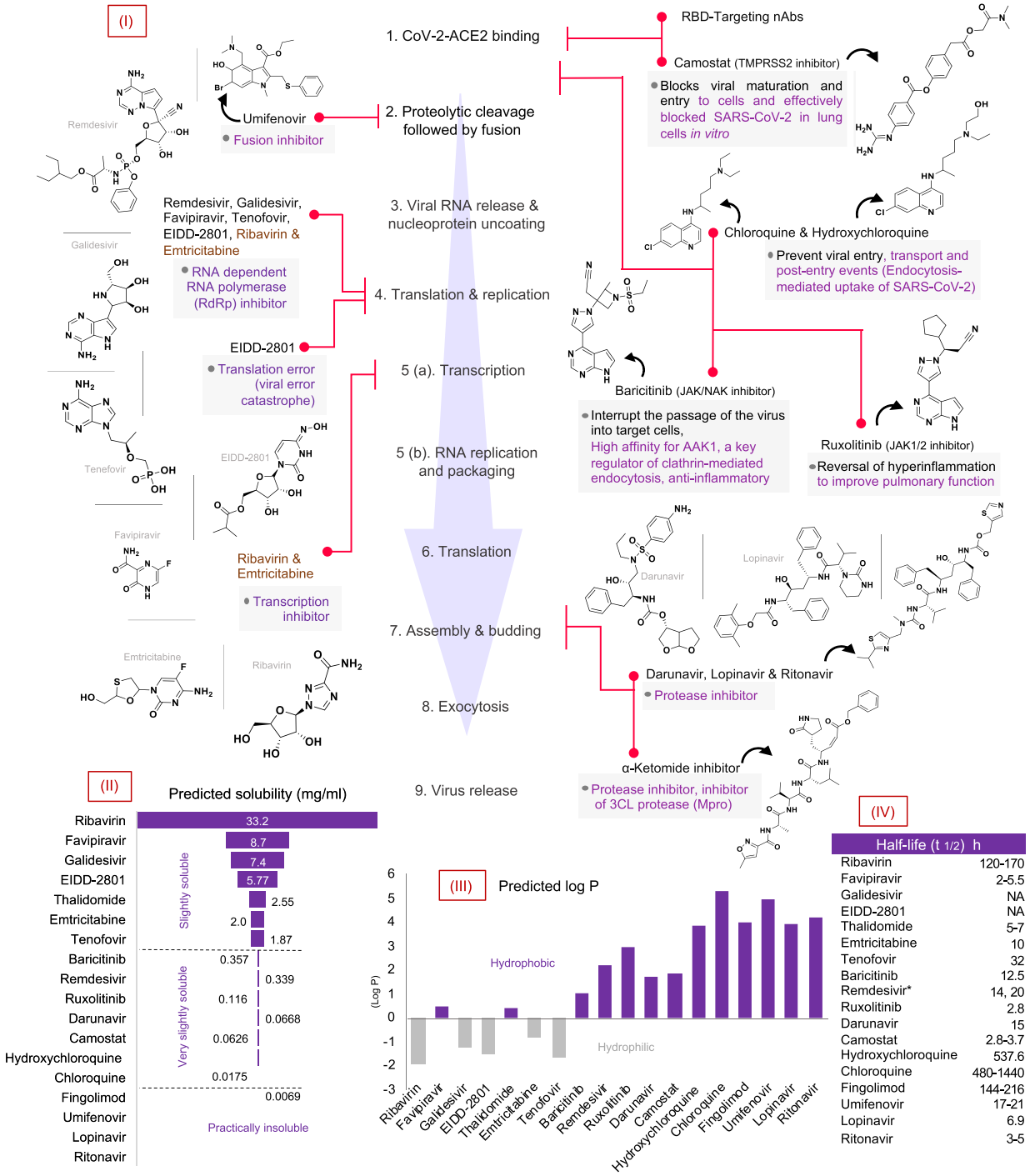

Figure 3. Existing antiviral molecules under development for COVID-19 therapeutics. (I) Chemical structure, mechanism and site of action, (II, III) water solubility, and log *P* calculated by ALOGPS.[91−93] (IV) Half-life ($t_{1/2}$) of these molecules; remdesivir has a plasma $t_{1/2}$ of 0.39 h after intravenous dose in nonhuman primates. Its metabolite nucleoside triphosphate has a $t_{1/2}$ of 14 h in nonhuman primates and approximately 20 h in humans.

further activating signaling pathways and nuclear translocation of transcription factors [like nuclear factor κB (NF-κB), interferon response factors (IRF3 and IRF7), and activator protein 1 (AP-1)].[53,54] These transcription factors further result in the initiation of tumor necrosis factors (TNF), chemokine-dependent inflammatory responses (through NF-κB and AP-1 stimulated expression), and type-I interferon (IFN-α and β)-dependent antiviral innate immune responses to suppress the viral infection.[55,56] On the other hand, adaptive immune responses are specific (for the virus or cells infected with the virus) and become noticeable after 1 week of the disease onset. The protective role of humoral (B-cell) immune response is primarily delivered by antibody secretion to neutralize the SARS-CoV-2 virus after specifically blocking the virus−host interaction.[46,57] T cell response against viral infection is significant for the direct killing of the infected cells (by $CD8^+$ T cells), whereas $CD4^+$ T cells help in cytokine production and priming of B cells and $CD8^+$ T cells.[58−60] Lisa

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and co-workers presented a chronological description of various events happening during SARS-CoV-2 infection (Figure 2).[61]

ORGAN SYSTEMS INVOLVED

SARS-CoV-2 primarily affects the respiratory system first and then spreads systemically to the heart, liver, and kidneys,[62] although it is still uncertain whether the viral infection directly results in organ or tissue injury as observed in COVID-19 patients. It is important to mention that ACE2 is highly expressed in the respiratory tract and other organs and tissues, encompassing the cardiovascular system (CVS), CNS, GIT, and female reproductive systems.[63,64] In the pulmonary system, there is a high expression of ACE2 in lung and bronchial branches cells, and it serves as the primary entry site and binding site for SARS-CoV-2.[65] This higher ACE2 expression makes the alveolar epithelial cells more accessible for SARS-CoV-2 and is clinically portrayed by the rapid development of pneumonia, advancing to the acute respiratory distress syndrome (ARDS) and multiple organ failure in severe cases.[10] In the CVS, cells (heart, endothelial cells, *etc.*) are known to show a high expression of ACE2, which acts to regulate blood pressure and myocardial contractility.[66] SARS-CoV-2 binding to ACE2 may result in the activation and upregulation of ACE2 downstream signal transduction pathways. This includes the activation of the Ras-ERK-AP-1 pathway, which further may activate the C−C motif chemokine ligand 2 (CCL2), which is a pro-fibrosis factor, resulting in the development of cardiac inflammation and fibrosis.[67] An increase in the levels of myocardial markers, specific (like hs-cTnI) and nonspecific (creatine kinase, creatine kinase MB isoenzyme and lactate dehydrogenase), has been reported in patients and can act as important tools for determining the level of COV-2 progression to the heart as well as other vital organs.[68] In the CNS, COV-2 can infect the CNS in four different ways: (i) Direct infection injury: This involves (a) the blood circulatory pathway: the virus passes through the increased permeability of blood−brain barrier (BBB) due to a cytokine storm; (b) neuronal pathway: sensory nerve endings are the major target for viral infection, which may result in anterograde or retrograde axonal transport by motor kinesins and dyneins.[69] (ii) Hypoxia injury: A result of virus infection of lung tissues, exudative inflammation (both in the alveolar and interstitial region), pulmonary edema, and related disorders. An increase in alveolar gas exchange disorders causes hypoxia in the CNS, leading to increased anaerobic metabolism in the mitochondria of brain cells. This hypoxia leads to CNS hypertension (headaches), edema (drowsiness), swelling of olfactory bulbs (loss of taste) and can cause severe damage to CNS.[70] (iii) Immune system: The infection of macrophages, microglia, and astrocytes can activate the immune cells in the brain, and the consequent cytokine storm can result in severe brain damage.[71] (iv) ACE2: SARS-CoV-2 interaction with the ACE2 expressed in the capillary endothelium may damage the BBB and facilitate the virus entry by attacking the vascular system.[72] GIT: The high expression of ACE2 and TMPRSS2 in intestinal epithelial cells including each organ (duodenum, small intestine, pancreas, and liver) of the gastrointestinal tract makes these cells and organs potential targets of COV-2. A recent study by Xiao F *et al.* detected viral RNA and nucleocapsid protein in gastric, duodenal, and rectal epithelia.[73] Reproductive system: ACE2 is also highly expressed in reproductive organs, especially in placenta, uterus, and fetal interface of pregnant women, in order to counteract preeclampsia. The presence of ACE2 in fetal tissue can also provide target sites for COV-2 binding, causing further morbidity and mortality.[74,75]

THERAPEUTIC DEVELOPMENT AND VACCINE DEVELOPMENT

Therapeutic Development. Therapeutic and prophylactic strategies to deal with existing and potentially upcoming coronavirus infections are under development. The symptomatic treatment approach is presently followed in the absence of an exclusive antiviral treatment against SARS-CoV-2. Artificial intelligence and other computation tools are currently in use to assist the lengthy process of drug discovery and development against this new pathogen.[76−78] Using recently available genetic information and protein structure modeling, several therapeutic strategies based on drug repurposing are projected for the immediate treatment of infected patients.[79−82] Target identification to halt the pathogenesis of the viral infection holds the key in this development. Viral protease (3CLpro and PLpro), host cell produced protease (TMPRSS2), RNA polymerase (RdRp), and the interaction site of viral S protein with host receptor ACE2 are among the major targets identified for repurposing already existing antiviral molecules and new small molecules under development (Figure 3).[83−90]

Targeting the SARS-CoV-2 surface S protein using neutralizing antibody (nAbs) is another strategy proposed.[46,94,95] The close similarity of SARS-CoV-2 with SARS-CoV allows for researchers to explore the cross-neutralizing activity of nAbs developed for SARS-CoV against SARS-CoV-2 infection.[96−99] The approach is mostly directed at utilizing currently available genomic information to synthesize immunogenic segments against S protein including its RBD.[96−111] Several traditional and advanced lead screening and lab/animal testing methods are considered to speed up this usually slow and challenging process. It is also suggested that a combination of nAbs may be required to guarantee prophylaxis.[112] Some other targets that are considered for neutralizing antibody development are based on the information allied to the already known SARS and MERS infections.[38] These targets include IL-6/IL-6R, CD16, immunoreceptor tyrosine-based activation motif (ITAM), Toll-like receptor 3 (TLR3), dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin (DC-SIGN), interferon γ-inducible protein 10 (IP-10/CXCL10), intercellular adhesion molecule 3 (ICAM-3), and monocyte chemoattractant protein-1 (MCP1).[51,113,114] Cytokine therapy (comprising of chemokines, interferons, and others) is also proposed owing to its potential to halt viral replication and thus its use in treating SARS infections.[114,115] Some examples include recombinant interferon (rSIFN-co), recombinant human interferon INF-ω (rhIFN-ω), polymer conjugate cysteine variants (MetIL-29C172S-PEG), long-lasting interferon fused with human serum albumin-binding peptide (HSA-IFN), and pegylated IFN-λ1.[116] Interferon-α2b treatment in COVID-19 patients resulted in the shortened duration of viral shedding, particularly the reduction of markers of acute inflammation such C-reactive protein and IL6.[117]

Targeting the SARS-CoV-2 viral RNA genome using RNA interference (RNAi) or antisense oligonucleotides is another interesting approach to consider.[118] Designing a small complementary RNA sequence to target and neutralize

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Table 1. The Most Advanced COVID-19 Vaccine Candidates Recently Moved to Clinical Development

candidate, lead developer, and clinical trial identifier number	status and details	design and characteristics	start and estimated completion date
mRNA-1273 (Moderna) (NCT04283461)	open label, open-label, phase I dose-ranging study to evaluate the safety and immunogenicity 45 participants	novel LNP-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized spike (S) protein of SARS-CoV-2	March 3, 2020 to June 1, 2021
Ad5-nCoV (CanSino Biologicals) (NCT04313127)	dose-escalating phase 1 study to evaluate the safety, reactogenicity and immunogenicity 108 participants	recombinant novel coronavirus vaccine (Adenovirus type 5 vector that expresses S protein)	March 16, 2020 to December 20, 2022
INO-4800 (Inovio Pharmaceuticals) (NCT04336410)	open-label study, phase I study to evaluate the safety, tolerability and immunogenicity 40 participants	DNA plasmid encoding S protein (intradermal administration followed by electroporation); device used: CELLECTRA 2000	April 3, 2020 to November 30, 2020
LV-SMENP-DC (Shenzhen Geno-Immune Medical Institute, China) (NCT04276896)	multicenter trial, phase I/II study to evaluate safety and efficacy of this LV vaccine (LV-SMENP) 100 participants	dendritic cells modified with engineered lentiviral vector expressing synthetic minigenes based on selected conserved and critical genomic structural and protease protein domains	March 24, 2020 to December 31, 2024
pathogen-specific artificial antigen presenting cell (aAPC) (Shenzhen Geno-Immune Medical Institute, China) (NCT04299724)	open-label study, phase 1 study to evaluate the safety and immunity 100 participants	aAPCs with lentivirus modification including immune modulatory genes and the viral minigenes based on domains of selected viral proteins	February 15, 2020 to December 31, 2024
ChAdOx1 nCoV-19 (COV001) University of Oxford, England (NCT04324606)	single-blinded, randomized, multicenter study, phase I/II study to evaluate the efficacy, safety, and immunogenicity; anticipated 1112 participants (4 study groups)	adenovirus vaccine vector (nonreplicating viral vector encoding the spike protein of SARS-CoV-2), vaccine will be administered intramuscularly	April 23, 2020 to May 2021
BNT162 Biontech/Fosun Pharma/Pfizer (NCT04368728)	randomized, placebo-controlled, observer-blind, dose-finding, and vaccine candidate-selection study, Phase I/II		April 29, 2020 to March 8, 2023
BNT162 Biontech (NCT04380701)	Study to evaluate the safety, tolerability, immunogenicity, and potential efficacy two-part, dose-escalation trial, A multisite phase I/II, investigating the safety and immunogenicity using different dosing regimens	LNP formulation-based mRNA vaccine (four different vaccine candidates, each representing different target antigens). Two candidates include a nucleoside-modified mRNA, one includes a uridine containing mRNA (uRNA), and one candidate utilizes self-amplifying mRNA (saRNA)	April 20, 2020 (starting date)

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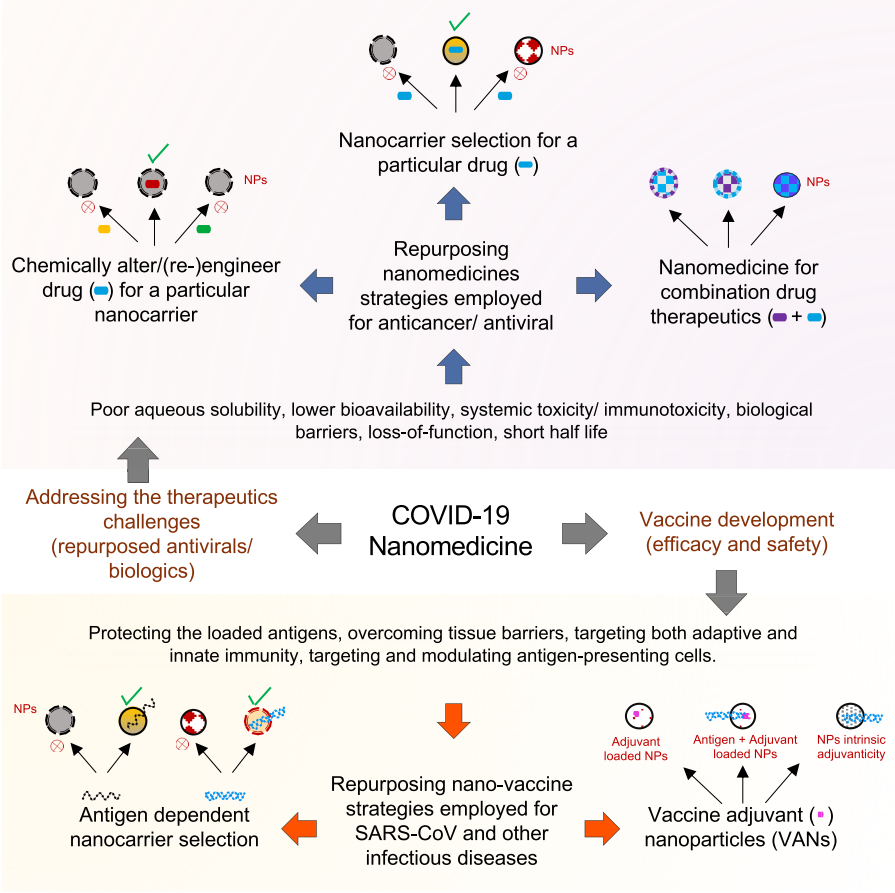

Figure 4. Nanomedicine strategies for COVID-19 therapeutics and vaccine development.

SARS-CoV-2 RNA is challenging because of the unidentified conserved RNA sequence domains for this virus. It is reported that RNAi such as small interfering RNAs (siRNAs), RNA aptamers, and antisense oligonucleotides might find use in treating the SARS infection.[119,120] Further within siRNA therapy, different regions are described as targets to halt the infection. siRNA-M1 and M2 were designed to precisely target regions of the SARS protein M mRNA with an interference efficiency of >70% (patent application CN101173275). Similarly, it was disclosed that a series of siRNAs were revealed to target and impede the expression of SARS-CoV M, N, and E genes. Other siRNA targets reported include RNA polymerase and replicase regions of coronavirus inhibiting its infection and replication. Antisense oligonucleotides and RNA aptamers are also reported to target the pseudoknot structure and unwinding of the SARS CoV RNA, respectively.[114]

Vaccine Development. The COVID-19 pandemic has severely impacted human lives, and desperate efforts are being employed across the world to develop safe and effective vaccines. The first vaccine candidate has already made it to human clinical trials as a result of fast-tracked development strategies and advanced vaccine technological platforms.[121] Similar to what was explained earlier for therapeutic development, the significant genomic match of SARS-CoV-2 with other coronaviruses is helping the vaccine developers to facilitate designs toward the most promising vaccine candidates. The target strategy for most of the vaccine candidates is to induce nAbs against the viral S protein, averting the ACE2-mediated host uptake. In the case of SARS-CoV vaccine development, higher nAbs titers and better protection were reported with S protein subunit vaccines when compared to any other target strategy. SARS/MERS vaccine development research suggests S protein subunits, RBD of the S1 subunit, and S protein/gene as the most preferred target sites.[122−124] But still, knowledge of SARS-CoV-2 specific antigen(s) for under trial vaccine candidates is limited. The development of COVID-19 vaccine candidates is relying on several high-tech platforms including attenuated and inactivated viruses, replicating and nonreplicating viral vectors, DNA and mRNA, virus-like particles, and recombinant protein-based approaches. Some platforms offer key advantages such as viral vectors with their strong immune response, superior protein expression, and prolonged stability, and DNA or mRNA offers antigen manipulation flexibility, whereas the recombinant protein-based development approach is easier to scale up using existing production capabilities. In Table 1 we list some of the most advanced COVID-19 vaccine candidates that have recently moved into clinical development.[125,126] Enhancing the immunogenicity using vaccine adjuvants is also under consideration to lower the viable dose and to widen the therapeutic and safety window.[127,128] Compromised immune systems and high risk of disease in the elderly population also demand adjuvant strategies to improve efficacy of vaccines in this age group.[129] Some licensed adjuvants developed specifically for COVID-19 vaccine are AS03 (GlaxoSmith-Kine), MF59 (Seqirus), and CpG 1018 (Dynavax).

Role of Nanotechnology. The COVID-19 crisis also demands an urgent analysis of all the available nanotechnology tools. While nanomedicine strategies are in use for the design of the vaccine carriers, there are not enough other nano-

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technology approaches being explored to tackle the current outbreak. This manuscript attempts to systematically present the current status of nanotechnology use in therapeutics and vaccine development. Therapeutic development and challenges against SARS-CoV-2 infection are not so different from other infectious diseases as well as oncology research.[130] Similarly, the vaccine development holds significant commonalities with strategies explored against previously known SARS, MERS coronaviruses.[121,131] Hence, it is worth revisiting these closely related therapeutic/vaccine strategies and associated nanotechnology use, and this way our aim is to design "repurposed nanotechnology" to fast-track the current research (Figure 4).

Antiviral and Biomolecular Delivery. Nanocarrier-based therapeutics offers several opportunities to address the limitations of current antiviral therapy. Some basic challenges such as poor aqueous solubility and low bioavailability can be solved by nanocarrier-based antiviral drugs delivery, modifying its pharmacokinetics/pharmacodynamics properties and resulting in dose reduction, reduced toxicity, and improved drug bioavailability and maintenance of the suppression of viral spread. Despite viral suppression in the plasma, another kind of challenge is the presence of subtherapeutic concentrations of an antiviral drug in reserve sites.[132,133] Active targeted nanocarriers offer the opportunity to cross biological barriers and attain therapeutic concentrations in sheltered viral reservoirs.[134] It is further possible to target a specific organ and cellular and intercellular sites involved in the pathophysiology of SARS-CoV-2 (possibly ACE2 expressing cells, domains of viral S protein, cathepsin binding sites, *etc.*). Controlled/sustained drug-releasing nanocarriers are the best solution to mitigate the risk effects of poor patient compliance and viral rebound during the treatment of viral infections. A nanomedicine strategy is thus a powerful tool to transform the antivirals repurposing and improving the COVID-19 therapeutic management.[135]

The role of nanocarrier technologies is decisive in the development of RNAi, nAbs, protein, peptide, and other biologicals.[136] An important prerequisite with such drug candidates is the protection against degradation "loss-of-function" in systemic circulation and intracellular delivery. Systemic toxicity/immunotoxicity is another big issue, more specifically with protein and peptide-based drugs. Nanocarrier-based delivery (including biologic prodrug) ensures improved half-life of the biologicals by preventing premature drug release and degradation, along with evasion of renal and hepatic clearance.[137] Engineered nanocarriers offer stealth characteristics to evade immune recognition and better cellular uptake. Targeted nanoparticles provide an improved rate of endocytosis which better ensures delivery of a therapeutic nanoparticle dose to the target cell. Ability to carry a higher drug load entails the delivery of fewer nanoparticles and is further supported with a controlled drug release within the cells ensuring reduced side effects.[138−143]

Nanomedicine Approach for COVID-19 Therapeutics (Rational Selection of Drug-Nanocarrier Combination). A broad range of active moieties including antivirals, biologics, and nucleic acids can be loaded and delivered by nanocarriers. Connecting the right therapeutic candidate to the right nanocarrier, aimed for a specific disease condition, is crucial for the commercial success of nanomedicine against the SARS-CoV-2 virus. This nanomedicine approach needs to deal with reformulating approved as well as under trial drug candidates to improve the "therapeutic index" (TI), predominantly by addressing the limitations associated with the drug molecule and mitigating the conventional toxicity or side effects. Therapeutic developments proposed for COVID-19 treatment possess a basic similarity with oncology research. Hence, the currently available nanomedicine platforms for anticancer therapeutics should be considered in order to nurture this approach.[144,145] Better mechanistic understanding of drug-specific side effects is the "first step" in this development process. The "second step" in this process is to scrutinize and select a very basic nanostrategy to maximize the impact of nanomedicine on the drug's TI. Nanodelivery of biologics also requires smart nanocarrier designs embracing strategies like the prodrug approach.[146,147] A rational development and clinical translation of nanomedicine will require several strategic track developments (fast paced and slower paced ones) tackling the right translational challenge and fostering more nanomedicine research.[148] Strategic guidelines proposed for the development of anticancer/antiviral nanomedicines are of high importance, emphasizing the right areas and precise tools to fast-track the COVID-19 nanomedicine research.

Strategy 1. Nanocarrier Selection to Bypass the Conventional Limitations of a Drug Candidate. For example, relatively safe antibody-drug conjugates of highly toxic auristatins are approved for the treatment of hematological cancers. A major limitation for use of these conjugates is the very low tolerable drug payloads. In order to solve this problem, polymeric nanoparticles were developed with a high auristatin payload to achieve efficient and safe tumor suppression.[149] Similarly, formulating poly(ethylene glycol)-poly(lactide)-based nanoparticles loaded with Aurora B kinase inhibitor revealed increased efficacy and reduced toxicity as compared to its free form which produced intolerable side effects in phase II clinical trials.[150] A well-known limitation of nucleic acid (*e.g.*, RNAi) drug candidates is their systemic circulation instability and the prerequisite of their intracellular delivery.[151−153] Lipid nanoparticles (LNPs) carrying siRNA are an example of a nanotechnology platform (Onpattro) used to prevent this systemic degradation along with the benefit of liver-targeting.[154] Lipid-coated mesoporous silica nanoparticles, used to deliver a highly hydrophilic and unstable antiviral molecule ML336 (chemical inhibitor of Venezuelan equine encephalitis virus), showed enhanced circulation time and biocompatibility of the ML336 *in vivo*.[155] Sago and co-workers reported a high-throughput method (named FIND) to screen LNPs that can bypass liver and deliver functional mRNA to cells *in vivo*.[156] Broadly, the relationship between nanoparticle structure and mRNA *in vivo* delivery targets may be elucidated from this study. Nanocarriers are used to prevent the systemic immunotoxicity of the protein-based drugs and promote immuno-oncology therapeutics.[157]

Strategy 2. Chemically Alter/(Re)engineer Drugs. Drug molecules are altered to improve their compatibility with a particular class or type of nanocarriers, rendering this a more generic approach for drug candidates with similar physicochemical properties.[158,159] Lipid bilayer nanocarriers (liposomes) are preferred nanocarriers for pH gradient-based remote loading of amphiphilic and ionizable drugs.[160] The hydrophobicity of doxorubicin was chemically modified to increase its compatibility with poly(lactic-*co*-glycolic acid) nanoparticles.[161] Another approach of interest here is the synthesis of "prodrugs" to ensure their compatibility and incorporation within particular nanocarriers along with their controlled and localized release characteristics. Anti-HIV

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Table 2. Combination Drug Treatments Proposed for COVID-19

combination description	candidates	status
protease inhibitors	ritonavir + lopinavir	under trial of COVID-19[186,187]
non-nucleoside reverse transcriptase inhibitor + nucleotide reverse transcriptase inhibitor	emtricitabine + tenofovir	under trial of COVID-19[188]
nucleoside inhibitor + protease inhibitor	ribavirin + ritonavir/lopinavir	clinical study of SARS[189] NCT00578825
antiretroviral protease inhibitor + cobicistat (to improve bioavailability and $t_{1/2}$)	darunavir + cobicistat	under trial of COVID-19[189]
antiviral + type I interferons - signaling proteins made and released by host cells during viral infections	IFN (α, β, IFNα2a or rIFN-α2b or IFN-β1a) + ribavirin	clinical study of SARS,[190] MERS[191,192]
interferons - signaling proteins made and released by host cells during viral infections + antiviral + steroid hormones	IFN + ribavirin + steroids	clinical study of SARS[193]
protease inhibitor + proteins made and released by host cells + antiviral	lopinavir + ritonavir + IFN + ribavirin	clinical study of MERS[194]
type I interferons - signaling proteins made and released by host cells during viral infections + immunosuppressant	IFN-β1a + mycophenolate mofetil	clinical study of MERS[195]
protease inhibitors + proteins made and released by host cells	lopinavir + ritonavir + IFNβ1b	clinical study of MERS[193]
synthetically developed recombinant type-I interferon + steroid hormones	IFN alfacon-1 + corticosteroids	clinical study of MERS[196]

prodrugs of antiretroviral (ARV) candidate cabotegravir has been synthesized by functionalizing fatty acid esters (with variable carbon lengths), followed by its poloxamer coating to get stable nanocrystal formulations. *In vivo* pharmacokinetic studies in mice and rhesus macaques revealed significantly improved effectiveness of cabotegravir, showing prolonged drug release and pharmacokinetic parameters.[162] Another ARV prodrug strategy for highly aqueous-soluble emtricitabine (using bioreversible carbonate and carbamate masking groups) shows sustained prodrug release predicted by *in vitro* to *in vivo* extrapolation modeling.[163] Wei and co-workers have reported the use of cholesterol-modified hydroxychloroquine (Chol-HCQ) loaded liposomes that lowered the dose and toxicity of hydroxychloroquine and also inhibited the proliferation of rat lung fibroblasts, thereby, reducing pulmonary fibrosis. This strategy can be adopted to have dual benefits in SAR-COV-2 patients, which show viral load and pulmonary fibrosis.[164] Using a hydrolyzable ester linkage, an irinotecan (hydrophilic) and chlorambucil (hydrophobic) anticancer drug−drug conjugate has been synthesized.[165] Nanoparticles synthesized by self-assembly of this amphiphilic drug−drug conjugate shows prolonged systemic retention, tumor tissue accumulation, and increased cellular uptake. Hydrolyzable ester linkers conjugated to docetaxel permitted its effective loading and release from core-cross-linked polymeric micelles to provide a high therapeutic efficacy against breast and ovarian cancer.[166] Another similar prodrug "fatty acids conjugated to cabazitaxel" with PEG-lipid results in self-assembled nanoparticles showing reduced systemic toxicity and superior anticancer efficacy.[167]

Strategy 3. Nanomedicine for Combination Drug Therapeutics. Combination drug therapy is another possibility for treatment of COVID-19, offering several advantages such as lower dosages of the individual drugs causing fewer side effects, achieving multiple and complimenting therapeutic targets, and reducing the likelihood of resistance development. Several such combinations for novel coronavirus treatment are documented in the WHO landscape information (Table 2). Nanocarriers are also intrinsically very useful for the delivery of multiple drugs with different physicochemical properties promising the full potential of combination therapies.[168,169] The flexibility offered by a variety of nanomaterials and fabrication techniques enables the design of drug combinations loaded in nanocarriers with excellent control in preserving synergistic drug ratios, overlapping pharmacokinetics, and reducing combination allied side-effects.[170] Various nanocarrier strategies are described for the co-encapsulation of both hydrophobic and hydrophilic drugs (Figure 5), achieving the sequential release of two drugs,

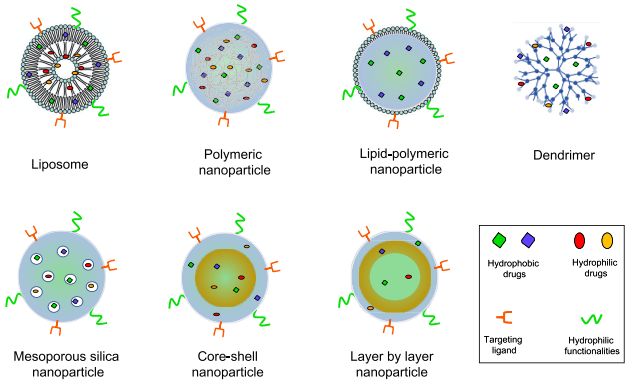

Figure 5. Nanocarrier platforms utilized for combination drug therapeutics.

ratiometric loading and controlled release of three drug candidates, codelivery of RNAi/plasmidDNA + chemotherapeutics, and codelivery of siRNA + micoRNA.[171−179] A nanosuspension of LNPs loaded with three ARVs drugs (two hydrophobic: lopinavir and ritonavir and one hydrophilic: tenofovir) has been formulated to overcome the lymph node drug insufficiency of the oral combination of these drugs. This nanoparticle formulation showed long-lasting plasma drug profiles and better lymph node drug levels in the macaques *in vivo* model.[180] A liposomal nanoformulation (Vyxeos) coloaded with a fixed combination of anticancer drugs daunorubicin and cytarabine was recently approved by US-FDA to treat acute myeloid leukemia in adults. Multidrug-loaded (antiretrovirals, latency reactivating agents, and drug abuse antagonist) pegylated-magneto-liposomal nanoformulations have shown *in vitro* and *in vivo* BBB transmigration with significant anti-HIV activity in primary CNS cells. This multifunctional nanotherapeutic strategy can be applied to target SARS-COV-2 that has migrated to the CNS.[181] However, drug combination regimens are a standard of care for a wide range of therapeutics, but optimizing their nanoformulations is an uphill task. These optimization challenges include analyzing the interaction between two or more drugs, balancing the antagonism/synergy/toxicity, and

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controlling the release profile of individual drugs.[182,183] High-throughput screening methods are required to understand the biological interactions and discover any kind of synergism that is present. *In vitro* screening methods to determine ideal drug ratios demand an upgrade to mimic the 3D microenvironment of the target human tissue.[184] Preclinical animal models are critical to accelerating the clinical translation, but a disparity between the model of disease in animals and human disease is the major reason for the failure of the study.[185] Nanomedicine scientists should take advantage of advanced drug development tools, screening technologies, bioinformatics, animal models, *etc.* to investigate and validate nanoparticle combination therapeutics.

Vaccine Delivery. The apparent similarity of SARS-CoV-2 with other viruses (mainly SARS-CoV and MERS-CoV), along with the previous knowledge of their protective immune responses, is of great help to successfully develop COVID-19 vaccine.[121,131] Nanoparticles can be loaded with a wide range of antigenic moieties (by physical entrapment or chemical conjugation), and a correct antigenic display makes it a highly relevant alternate in vaccinology when compared to conventional approaches.[197−199] In addition to safeguarding the native structure of the antigen, nanoparticles also improve the delivery and presentation of antigens to the antigen-presenting cells (APCs). The key advantages of vaccine nanocarriers are their nanosize, since many biological systems such as viruses (including SARS-CoV-2) and proteins are also nanosized. Nanoparticles can be administered by oral and intranasal routes and subcutaneous and intramuscular injections, offering a key advantage by overcoming tissue barriers and targeting key locations such as lymph nodes, penetrate mucosal, and epithelial barriers (airway, nasal, gastrointestinal, *etc.*).[200−202] Previous reports have suggested that both humoral and cell-mediated immunity performs a protective role in the SARS-CoV infection.[203,204] Nanoparticles have shown their ability to target both adaptive (T cells, B cells) and innate immune systems (macrophages, monocytes, neutrophils) at the cellular level. Modulating APCs using nanoparticles could be very important, particularly for COVID-19 vaccine strategies.[205,206] The ability of nanoparticles to deliver antigen to dendritic cells (DCs) by enhancing antigen presentation and several other mechanisms can promote T cell immunity.[207] Smith *et al.* explained various nanoparticle-based mechanisms to alter the immune response induction in (Figure 6).[208] To improve the efficacy and safety of the vaccine approach, a big advantage presented by nanoparticles is their ability to deliver molecular adjuvants, and, in some cases, nanomaterials themselves possess an intrinsic adjuvant property for the loaded antigens. The WHO reports (dated May 27, 2020) various preclinical stage nanoparticle-based vaccine candidates (Table 3).[126]

Nanomedicine Approach for COVID-19 Vaccine. Overall vaccine history indicates major successes against acute infectious diseases, where naturally developed immunity (majorly by neutralizing antibodies) provides enduring protection in a section of patients. One of the bigger challenges in the COVID-19 vaccine research is to identify approaches that stimulate both the T cell and B cell immunity against this virus. Another challenge is the necessity of accelerating the development of precise "next-generation" vaccine strategies that may also address specific population subgroups or individuals with compromised immunity.[209] Smart strategies to develop nanocarrier-based COVID-19 vaccines are equally important and sometimes overlapping

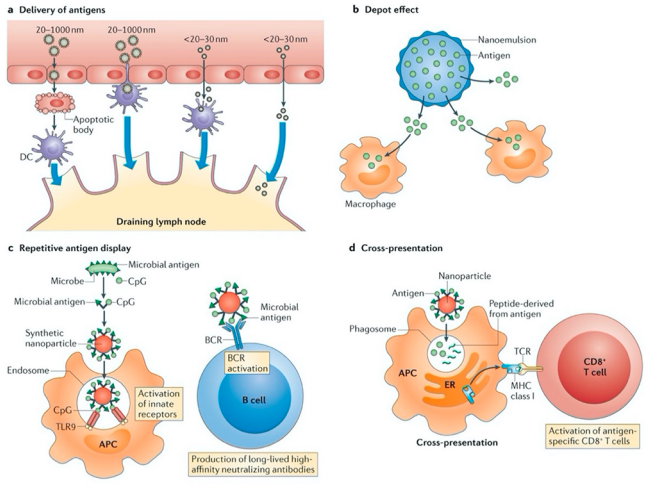

Figure 6. Nanoparticle-based immune response modulation. (a) Antigen delivery by nanoparticles (size-dependent penetration and tissue or organ targeting). (b) Depot effect provides a prolonged and sustained release of stable antigen. (c) Repetitive antigen display as a result of the antigen presentation on the nanoparticle surface assists the receptor activation on APCs and B cells and (d) cross presentation of the antigen delivered by the nanoparticles (cytosolic delivery) to activate antigen specific $CD8^+$ T cells. Antigen-presenting cell (APC); dendritic cell (DC); endoplasmic reticulum (ER); B cell receptor (BCR); T cell receptor (TCR). Adapted with permission from ref 208. Copyright 2013 Springer Nature.

Table 3. Nanoparticle-based vaccine candidates in preclinical evaluation Mentioned in the DRAFT Landscape of COVID-19 Candidate Vaccines (as of May 27, 2020)

platform	type of candidate vaccine[a]	developer
protein subunit	nanoparticle vaccine + matrix M (adjuvant) (based on recombinant SARS-CoV-2 glycoprotein)	Novavax
	peptide antigens formulated in LNPs formulation	IMV, Inc.
	nanoparticle vaccine (recombinant protein) (S protein and other epitopes based)	Scientific Research Institute of Vaccines and Sera, Saint Petersburg
	Nanoparticle vaccine	LakePharma, Inc.
RNA	LNPs formulation of mRNA	Sanofi Pasteur/Translate Bio
	LNPs-encapsulated mRNA cocktail encoding VLP	Fudan University/Shanghai JiaoTong University/ RNACure Biopharma
	LNPs-encapsulated mRNA encoding RBD	
	LNP-encapsulated mRNA	University of Tokyo/ Daiichi-Sankyo
	liposome- encapsulated mRNA	BIOCAD

[a]LNPs: lipid nanoparticles, VLP: virus-like particle.

when paralleled to nanocarrier-based therapeutics.[121,210] The nanovaccine strategy also requires a strong focus on the cellular presentation of the selected antigen, along with the selection of appropriate nanocarrier/nanomaterial to induce complimenting immunomodulatory effects. The following section highlights the rational design of nanocarrier-based vaccines with two strategies.

Strategy 1. Antigen-Dependent Nanocarrier Selection. Loading antigens inside or on the surface of nanocarriers is dependent on several factors including the antigen's physicochemical characteristics, biological stability, target sites, and required immunogen release rate. Physical

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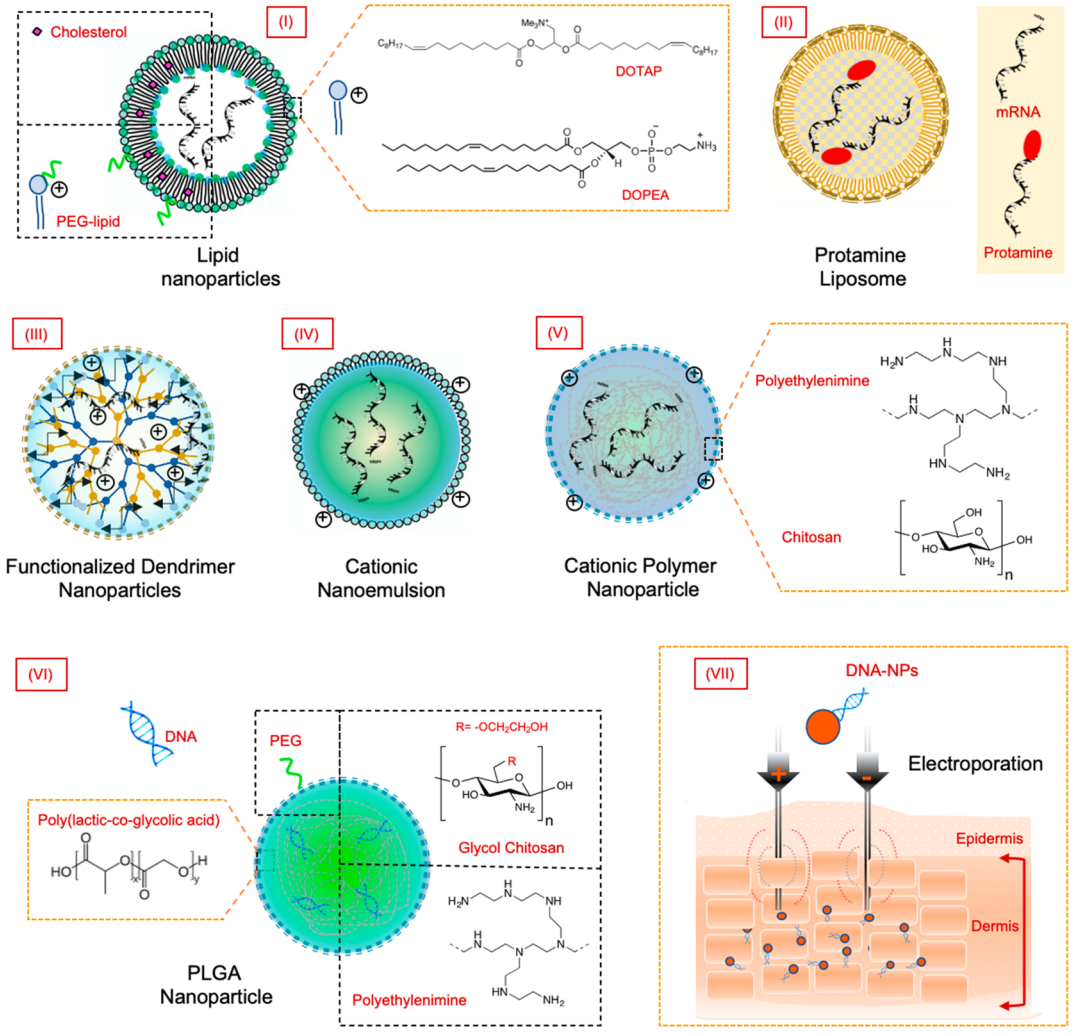

Figure 7. Major delivery methods for mRNA and DNA vaccines. (I–V) Nanocarrier for mRNA delivery, (VI) nanocarriers for DNA delivery, and (VII) electroporation technology for the intradermal delivery of DNA vaccines.

adsorption of antigens on nanoparticles is based on its surface charge and noncovalent hydrophobic interactions. Antigens with an amphoteric nature are most suitable for adsorption or surface immobilization on nanocarriers such as chitosan and dextran sulfate-based polymeric nanoparticles, inorganic nanoparticles (such as AuNPs), and carbon nanotubes.[211−214] Antigen release in such cases is predesigned based on the properties of the biological environment like pH, ionic strength, temperature, *etc.* Encapsulation and matrix entrapment of the antigens within a nanocarrier is another technique used to prevent its biological degradation. Poly(lactide-*co*-glycolide) (PLGA) nanoparticles are ideal for encapsulating antigens and provide controlled or extended biological release.[215] These nanoparticles are effective preclinically in carrying antigens such as HBsAg, malaria antigens tetanus toxoid, *Listeria monocytogenes* antigens, and *Bacillus anthracis* spores, generating prolonged cellular and humoral immune response.[216]

The mRNA-based COVID-19 vaccine is already under clinical trial employing LNPs as a carrier. Naked mRNAs are sensitive to the degradation by extracellular RNases, thus formulating its delivery vehicle is essential.[217,218] Further, these mRNAs entail their cell-specific receptor recognition and lipid membrane penetration. Cytosolic presence of exogeneous mRNA then triggers the cellular machinery for its translation into fully functional protein.[219] LNPs are virus-sized (80−200 nm) particles synthesized by the self-assembly of an ionizable cationic lipid.[220] They possess the ability to deliver mRNA efficiently into the cytoplasm, as demonstrated by several studies. Sustained-release kinetics of mRNA expression and thus protein translation can be achieved by opting for intramuscular and intradermal routes, providing high antibody titers, and both B cells and T cells immune responses.[138] Different nanoparticles of these cationic lipids (such as 1,2-dioleoyloxy-3-trimethylammoniumpropane (DOTAP) or dioleoylphosphatidylethanolamine (DOPE)) are formulated with subtle modifications (such as cationic lipids + cholesterol nanoparticle, cationic lipids + cholesterol + PEG-LNP), where cholesterol is used to increase stability and PEG-lipid to increases the formulation half-life. Apart from LNPs, other mRNA nanocarriers include protamine (cationic peptide) nanoliposomes (∼100 nm), PEG-lipid functionalized dendrimer nanoparticles (∼200 nm), positively charged oil-in-water (O/W) cationic nanoemulsion (∼120 nm), polyethylenimine nanoparticles (100−300 nm), and cationic polymer (chitosan) nanoparticles (300−600 nm).[221−223] Figure 7, I−V represents the mRNA vaccines delivery methods and nanocarriers commonly used.

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Similar to mRNA, naked DNA also experiences systemic degradation by nucleases and incomplete delivery to specialized immune cells. Nanocarriers based on cationic lipids (quite similar to mRNA deliver), synthetic and natural polymers, and inorganic particles are proposed for DNA-based vaccine formulations. Polymeric nanocarriers encapsulating DNA prevent biological inactivation and provide controlled release and targeted cell delivery. PLGA nanocarriers are the most studied polymeric platform for DNA vaccine development, showing improved systemic antigen-specific antibody responses.[224,225] To improve the efficiency of DNA loading and systemic protection, functional or composite PLGA nanoparticles (such as cationic glycol-chitosan + PLGA, PLGA+ polyethylenimine (PEI)) are explored (Figure 7, VI).[226,227] Other well documented cationic polymer-based nanocarriers for DNA vaccine design are chitosan nanoparticles and PEI nanoparticles/complexes. Use of PEG functionalization on nanoparticle surfaces is quite common to introduce stealth characteristics (it renders them undetectable to phagocytes and prevent reticuloendothelial system clearance), prevent nonspecific protein interaction, reduce systemic toxicity, and improve stability.[228,229] To improve the delivery of mRNA/DNA across the cell and nucleus membrane, physical technologies such as the gene gun and electroporation are being explored. Currently, vaccine development is taking advantage of electroporation technology to induce pores in the cell membrane to insert the DNA (Figure 7, VII).[215,230,231] Surface electroporation DNA coated-PLGA nanoparticles have shown efficient cellular delivery to elicit B cell and T cell response in pigs.[231] The clinical future of such portable electroporation technologies is now apparent in the race of COVID-19 vaccine research.[232] An ongoing clinical trial (NCT04336410) is using a DNA plasmid encoding SARS-CoV-2 S-protein as a vaccine candidate for intradermally administration using an electroporation device (CELLECTRA 2000).

Strategy 2. Vaccine Adjuvant Nanoparticles. Vaccine adjuvants nanoparticles (VANs) are considered to improve the overall efficacy and safety of the generated immune response. Particularly in COVID-19 pandemic situation, vaccine adjuvants are critical to reducing the required antigen dose (dose-sparing), permitting the production of more units and making it available to larger population.[233] Among many preclinical COVID-19 vaccine candidates, five protein subunit vaccine candidates are reported using a combination of antigen and adjuvant. NVX-CoV2373, a nanoparticle vaccine (recombinant SARS-CoV-2 glycoprotein based) with an adjuvant (matrix M) is now expected to move into clinical trials soon. Hence, it is important to discuss the possible strategies employed by VANs in other research studies that could help to improve current COVID-19 vaccine designs. Informing specific immune cells to mount a protective immune response against a specific antigen is the basic mechanism of VANs designed to improve efficacy (by serving as immunity promoting cues, also called as "danger signals").[234] In the case of a virus, these danger signals are characterized as PAMPs and damage-associated molecular patterns (DAMPs) derived from the same virus.[235] PAMPs and DAMPs are recognized by specific receptors called pattern recognition receptors (PRRs). An example of such receptors is Toll-like receptors which are expressed by immune cells to upregulate robust T and B cell priming by releasing inflammatory cytokines.[236−238] Adjuvants improving safety provide a kind of counter-regulatory signal instructing the immune system to develop a tolerance for incoming antigens. VANs can either act as a nanocarrier for molecular adjuvants or have an inherent physicochemical property to stimulate pro- or anti-immunity pathway.[211]

VANs are designed to tackle the limitations related to the conventional delivery of molecular vaccine adjuvants such as rapid bloodstream clearance, systemic distribution, and lack of immune cell targeting as well as lack of antigen-adjuvant colocalization. Polymeric nanoparticles encapsulating small molecules are employed for lymphoid organ-specific delivery with controlled exposure. The dose-sparing effect is reported with antigen and cyclic dinucleotide (adjuvant; agonist of INF gene stimulator) coloaded liposomal nanoparticles showing safe and uncompromised immune responses.[239] Lymph node targeting of VANs is an established strategy to achieve a significantly high-dose-sparing effect, whereas DCs targeting VANs may enhance its adjuvanticity. *In vivo* study results against infectious challenge has shown PLGA and calcium phosphate nanoparticles co-encapsulating both antigen and adjuvants to improve efficacy by enhancing antigen uptake, APC activation, and higher antibody titers.[240−242] In other studies, co-encapsulation strategy allows the colocalization of antigen and adjuvant in endosomal/phagosomal compartments fostering the activation of DCs and triggers robust cross-presentation and T cell priming.[243,244] Synergized activation of APCs and prolonged antibody response was observed with the codelivery of TLR4 and TLR7 small molecule adjuvants using PLGA nanoparticles.[245] VANs (including PLGA, AuNPs) are also employed to codeliver self-antigens or immunoregulatory drugs as adjuvants to induce antigen-specific peripheral tolerance of autoreactive T cells and block any serious autoimmune response.[246−251]

Nanoparticles because of their intrinsic adjuvanticity (by activating complement system, inducing autophagy and activation of inflammasome) are also considered as VANs.[252−258] Surface chemistry and hydrophobicity of nanoparticles along with other physicochemical properties are capable of electing these adjuvanticity mechanisms intrinsically.[253,259,260] Hydroxyl groups dependent compliment system activation followed by cellular immunity enhancement is reported with pluronic-stabilized poly(propylene sulfide) nanoparticles.[259] Antigen conjugated alumina nanoparticles have been reported to enhance cellular and humoral immune responses as a result of autophagy induction in DCs, fostering antigen cross-presentation to T cells.[255] Gold and PLG nanoparticles are reported to activate NALP3 inflammasome in DCs, resulting in improved adjuvanticity similar to an alum-mediated adjuvanticity mechanism.[261,262] Increased side-chain hydrophobicity of poly(γ-glutamic acid) nanoparticles displayed augmented uptake and DCs activation.[263] Similarly, AuNP surface hydrophobicity can increase the expression of inflammatory cytokines both *in vitro* and *in vivo*.[264]

Vaccine adjuvants have been used to increase the efficiency and the antibody responses of vaccines in the elderly. They comprise the most vulnerable groups of the population and have the highest case-fatality rate of the COVID-19 disease.[265,266] Aging is associated with continuous chronic subclinical systemic inflammation (inflamm-aging) and acquired immune system weakening, that is, immune senescence.[267] Immune senescence is flagged with a significant decrease of immunoglobulin M, interferon levels, T-cell count, rate of cell division and proliferation, chemotaxis of neutrophils, and phagocytosis.[267,268] O/W emulsion, immune

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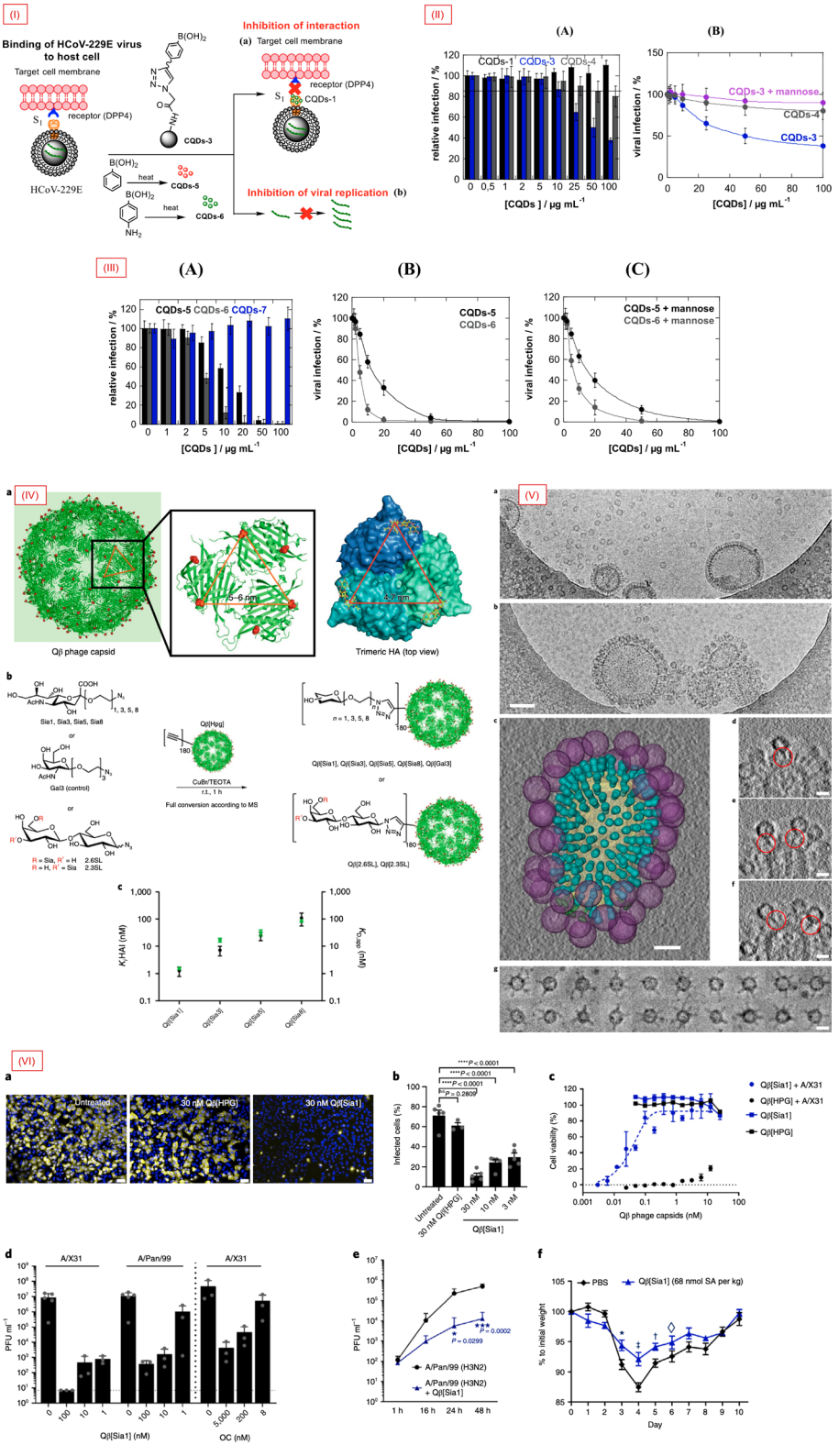

Figure 8. Miscellaneous nanotechnology approaches. (I) Hydrothermal synthesis of functionalized CQDs, as an inhibitor of HCoV-229E - Huh-7 cells (host cell) binding and further infection. (II, A) Concentration-dependent viral inhibition with CQDs (1−4) and (II, B) EC_{50} for CQDs (3, 4) and CQD-3 + mannose (incubation ratio of 2:1, 4 °C for overnight). (III, A) Concentration-dependent viral inhibition with CQDs (5−7), (III, B) EC_{50} for CQDs (5 and 6), and (III, C) EC_{50} for CQD (5 and 6) + mannose (incubation ratio of 2:1, 4 °C for overnight).[273] Adapted with permission from ref 273. Copyright 2019 American Chemical Society. (IV) Q*β* phage capsid as a multivalent and high affinity influenza A virus binder (a) structural resemblance between the Sia attachment sites (present on the capsid) and the HA-Sia binding pockets (on A/X31 virion), (b) functionalization procedure of Q*β* phage capsids to introduce Sia ligands, (c) haemagglutination inhibition assay against different HA units (K_iHAI, in black) and the apparent dissociation constants ($K_{D,\,app}$ in green) measured by microscale thermophoresis against A/X31 virion. (V) Cryo-TEM images showing diverse Q*β* capsids covering the A/X31 envelop and

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Figure 8. continued

blocking the host interaction: (a) Qβ[Gal3] with no virus interaction, (b) Qβ[Sia1] decorated with virus, (c) a 3D model showing multiple Qβ[Sia1] capsids (purple) attached with a single virion (yellow envelop), HA (cyan), and neuraminidase (NA, green) (scale for a, b: 100 nm and c: 25 nm). (d–f) Red circle indicates specific binding incidents of Qβ[Sia1] capsid to HA trimers and (g) binding events of viral HA ectodomains to discrete Qβ[Sia1] capsid presented with collection of 20 images (scale for d–g: 20 nm). (VI) Inhibition study of influenza A virus strains by Qβ[Sia1] capsid. (a) Confocal images showing Qβ[Sia1] capsids inhibiting viral infection (A/Pan/99) of A549 cells (infected cells: yellow, nuclei: blue and scale: 40 μm). (b) The percentage of infected cells (using viral nucleoprotein signal) with different treatments and a control. (c) Inhibition study of A/X31strains infection using Qβ[Sia1] phage capsid and its cell toxicity in the absence of virus (Qβ[Hpg] is used as control). (d) Bar graph showing the cell supernatant titers of A/X31 and A/Pan/99 viruses after the Qβ[Sia1] and oseltamivir carboxylate (OC) treatment (with no treatment as control, PFU: plaque forming units). (e) *Ex vivo* experiment showing the potential of Qβ[Sia1] capsid to inhibit the A/Pan/99 virion infection in human lung tissue. (f) *In vivo* experiment in BALB/c mice shows the potential of Qβ[Sia1] capsid to protect the A/X31 infections. Adapted with permission from ref 277. Copyright 2020 Springer Nature.

stimulating complexes, cationic and anionic liposomes, virosomes, and microparticles are among the various adjuvant's technologies developed to improve the influenza vaccination in the older population.[129] Squalene-based O/W emulsion adjuvants MF59 and AS03 have been licensed for influenza vaccines meant for the elderly.[269,270] A liposome-based adjuvant AS01 is another key example of licensed technology developed for the herpes zoster subunit vaccine aiming old age population (70 years or above).[271,272] Addition of adjuvants has shown a decreased risk of pneumonia and influenza in clinical trials and can hence play a significant role in regulating the immune system responses of the elderly, which further can be tuned for COVID-19 vaccine progress.[129]

SCOPE OF MISCELLANEOUS NANOTECHNOLOGY APPROACHES

The scope of nanotechnology for COVID-19 therapeutics and vaccine research is not limited to conventional therapeutic and vaccine designs. Several other approaches including advanced nanomaterial and biomimetic approaches represent good potential usage in a COVID-19-like outbreak. Szunerits and co-workers investigated the prospect of functionalized carbon quantum dots (CQDs) to inhibit the human coronavirus (HCoV-229E) infection (Figure 8, I–III).[273] CQDs of different sizes (<10 nm), surface potential (−7.9 to −39.2 mV), and functionalities were explored as inhibitors of Huh-7 cells (host cell) infection by HCoV-229E, and they showed a concentration-dependent virus inactivation. Boronic acid-modified CQDs showed the maximum efficacy with an EC_{50} value of 5.2 ± 0.7 μg mL^{-1}, illustrating the significance of boronic acid functionality to inhibit the early stage interaction of viral S-protein receptor with the host cell membrane. Cell membranes mimicking nanodecoys are an interesting choice to fool and trap pathogens. These biomimetic nanodecoys include liposomal formulations, reconstituted lipoproteins, and cell-membrane nanostructures.[274] Targeted surface engineered liposomes with antiviral antibodies constitute an effective strategy to provide protection against the infection of coxsackie A-21 virus.[275] Similarly, mosquito host-cell-membrane-wrapped nanodecoys are employed to trap the Zika virus and effectively prevent host cell infection.[276] Lauster and co-workers presented an interesting approach employing an influenza A virus spike-protein (hemeagglutinin, HA) mimicking a multivalent binder that can bind to the virus in a distinct multivalent mode and inhibit its infection.[277] Normally a multivalent manner binding is observed between the viral trimeric HA and the terminal sialic acid (Sia) residues of the host cell's surface glycans. Structurally defined presentation of Sia ligands are functionalized on a compact symmetrical 3D scaffold, that is, a bacteriophage capsid resembling a host cell and targeting the trimeric HA ectodomain of the virus. K16 residues present in the protein coat of symmetric icosahedral bacteriophages Qβ capsid (~25 nm diameter) provided an ideal platform to anchor Sia ligands with a varied length of the linker to mimic the HA trimer's binding sites (Figure 8, IV). Cryo-electron tomography showed these Qβ capsids covering the A/X31 virus (H3N2 subtype) envelop and significantly blocking the host cell interaction (Figure 8, V). Phage capsid nanoparticles have shown the potential to inhibit virus infection during *in vitro*, *ex vivo*, and *in vivo* studies (Figure 8, VI).

CONCLUSION AND OUTLOOK

Nanotechnology tools can play a pivotal role in advancing COVID-19 treatment and vaccine development. Information related to the structural morphology of the SARS-CoV-2 virus, its pathophysiology, and related immunological response is vital for nanotechnology scientists. In the absence of a specific antiviral against SARS-CoV-2, present therapeutics target the multifaceted molecular interactions involved in viral infections and majorly comprises repurposing already existing antiviral molecules used for other RNA viruses. Now, it is equally important to look for a suitable nanocarrier delivery technology to make these repurposed therapeutics safer and more effective. This manuscript provides systematic information on nanomedicine strategies employed to deliver small molecules, biologicals (specifically RNAi), and various combination therapies. Some strategies are also proposed for the rational development of this nanomedicine approach and its clinical translation. The journey of COVID-19 vaccine development is very impressive and involves high-tech platforms such as viral vectors, antigen carriers, and delivery technology. A mRNA-based vaccine employing nanoparticle (LNPs) delivery is already in clinical trials, whereas another vaccine candidate in the clinical phase is using electroporation technology for the intradermal administration of DNA plasmid. Since most of the COVID-19 vaccine candidates are sophisticated biological moieties (DNA, mRNA, recombinant proteins, engineered APCs, *etc.*), the scope of nanocarrier delivery becomes highly pertinent. This manuscript details the opportunities presented by these nanocarriers to potentiate the success of COVID-19 vaccine in terms of efficacy and safety. Some of these opportunities include nanocarrier-based effective/targeted delivery, better antigen presentation, and the induction of complimenting immunomodulatory effect. Rational designing of nanocarrier-based vaccine is equally important for its clinical success, hence strategies related to the nanocarrier selection for antigen loading and effective delivery

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are discussed in this manuscript. A special emphasis is given to VANs, which either act as a carrier for molecular adjuvants or mount a pro-or anti-immunity effect by their own. Some miscellaneous technologies based on functionalized CQDs and biomimicking nanoscaffolds are also discussed to present the scope of unconventional therapeutics. In this era of advanced nanoscience, we have all the tools to implement these technologies and play a frontline role in tackling this outbreak.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.0c04006.

S-Figure 1: Representation of the residues that are involved in the interaction of RBD domain of the SARS-CoV-2 with peptidase domain of Human ACE 2 (PDB ID: 6M0J). Table S1: Side effects/toxicity of existing antiviral molecules under development for COVID-19 therapeutics. Table S2: Predicted properties of existing antiviral molecules under development for COVID-19 therapeutics (PDF)

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G.C. designed and wrote the main manuscript text. S.K., V.C., and D.G. contributed to the specific sections. S.O.M.C. and M.J.M. supervised and directed the overall project. All authors contributed to the data analysis, compilation, and reviewed the manuscript.

Notes

The authors declare no competing financial interest.

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VOCABULARY

COVID-19, Coronavirus disease 2019, where SARS-CoV- 2 is the causative agent responsible for the COVID-19 pandemic; **angiotensin converting enzyme 2 (ACE2)**, a receptor, the host cell receptor responsible for SARS-CoV-2 viral entry into cells; **receptor binding domain (RBD)**, domains present in in the S1 subunit of the viral S protein responsible for its entry into the host cells by binding to a host receptor (ACE2); **pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)**, small molecular motifs derived from the virus, recognized by pattern-recognition receptors, which play a key role in innate immunity in the recognition of virus or drive inflammation in response to infections; **vaccine adjuvant nanoparticles (VANs)**, nanoparticles to enhance the overall "efficacy" and "safety" of a vaccine by various mechanism

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